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Neurosporacrassa, the orange bread mold has become a model system for the study of fungal genetics including sexual and asexual cycles. To facilitate the molecular studies in N. crassa, two expression vectors, pBL-ARS1 and pBL-ARS2, were constructed. The common features of the plasmids are: 1) the multiple cloning sites (MCS), 2) the inorganic sulfur-inducible ars promoter sequence and the qa-4 terminator sequence, 3) a partial his-3 gene sequence for homologous integration into the his-3 locus by recombination and 4) pUC19 base. The multiple cloning sites contain unique restriction endonuclease digestion sequences for *MscI*, *EagI*, *NsiI*, *SpeI*, *NotI*, and *SmaI*. While pBL-ARS1 has a Kozak sequence and an initiation codon in front of MCS, pBL-ARS1 does not have them.

F303

Molecular Analysis of *vanA* Gene Cluster in Vancomycin Resistant Enterococci (VRE) by Using PCR Amplification

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Recently infections of vancomycin resistant enterococci containing *vanA* gene have been increasing worldwide. The genes encoding the VanA type are located on mobile DNA elements, and conjugative transfer from VRE to the other enterococci may exist. We wanted to know about the distribution of the resistance genes and *vanA* gene cluster types in Korea. The internal and structural regions of *vanA* cluster were analyzed by using PCR fragment length polymorphism with 50 *E. faecium*, 12 *E. feacalis*, 3 *E. casseliflavus*, and 1 *E. gallinarum* isolated from clinical specimens. For *vanR* (645 bp), *vanS* (1,094 bp), *vanH* (943 bp), *vanA* (1,029 bp), *vanX* (424 bp), *vanY* (866 bp), and *vanYZ* (336 bp) intergenic regions, PCR products of expected size were obtained from *vanA* cluster (Tn1546). But for the *vanXY* and *vanSH*, amplicons of variable size were observed. For examples, the *vanXY* amplicons of 554 bp were obtained from 16 isolates and those of approximately 1,500 bp from 50 isolates. While 65 isolates produced amplicons of expected size (311 bp) for the *vanSH*, one produced about 1,900 bp amplicon. Therefore, dissemination of the resistance genes carried on transposable elements may be of greater importance than clonal spread.

F304

RFLP Pattern's of the IGS Region in *Fusarium* Species.

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The intergenic spacer (IGS) region, which is located between the 3′ end of 28S ribosomal DNA (rDNA) and the 5′ end of 18S rDNA, of *Fusarium* species was investigated using polymerase chain reaction-restriction fragment lenghth polymorphisms (PCR-RFLPs). The intergenic spacer of the rDNA is highly polymorphic, so provide useful tools for fungal taxonomic and phylogenetic studies. To investigate the genetic relatedness among 21 strains belonging to *F. graminearum*, *F. oxysporum*, *F. sambucinum*, and *F. solani*, after the IGS

region of the rDNA were amplified using the PCR and digested with 10 restriction enzyme, RFLP patterns were analyzed. The PCR primer CNL12 and CNS1 were used to amplify the IGS region. The restriction enzyme used were BglII, HincII, HindIII, EcoRI, NruI, SalI, SmaI, PstI, XbaI, XhoI. The size of amplification product showed the interspecific polymorphism is approximately 2350 bp for strains of *F. sambucinum*, 2600 bp for formae speciales of *F. oxysporum*, approximately 2700 bp for strains of *F. graminearum* except *F. gra.* UBC830 (2600 bp) and for *F. solani* except *F. sol. piperis* (2600 bp) and *F. sol. pisi* (2900 bp). Cluster analysis based on the presence and absence of comigrating restriction fragments devided into two major groups. One group is included *F. oxysporum* formae speciales, *F. gra.* UBC830, and *F. sol. piperis*. A second group is included strains of *F. graminearum*, *F. sambucinum*, and *F. solani*.

F305

Pseudomonas syringae pv. actinidiae Strains Isolated from Korea Produce the Phytotoxin Coronatine instead of Phaseolotoxin

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Pseudomonas syringae is divided into 57 pathovars and produces a wide spectrum of phytotoxic compounds. *P. syringae* pv. actinidiae was known to cause bacterial canker on kiwifruit. Many research have already confirmed that pv. actinidiae produces phaseolotoxin as a phytotoxic compound. *P. syringae* pv. actinidiae strains were collected from the major field of kiwifruit cultivation areas in Korea. These strains, together with type strain of this pathovar, were tested for the presence of several toxin related genes by using PCR. All Korean strains contained the gene involved in coronatine biosynthesis, whereas type strain of this pathovar possessed the gene for phaseolotoxin. The nucleotide sequence of PCR product of Korean strains was identical to that of *P. syringae* pv. glycinea. The location of the gene cluster was determined for coronatine producing Korean strains by subjecting their DNA to pulsed-field electrophoresis and Southern blot analysis with a hybridization probe from the coronatine gene cluster. The coronatine gene cluster was contained in plasmids with different size in all Korean strains tested. These results suggested that *P. syringae* pv. actinidiae isolated from Korea is not closely related to type strain of this pathovar.

F306

Nucleotide Sequence, Mutational Analysis and Expression of the Inducible Nickel Resistance Determinant from pEJH501 of *Hafnia alvei* 5-5

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The nucleotide sequence of the 4.8 kb *Sal* I -*Eco*R I fragment encoding nickel and cobalt resistance on plasmid pEJH501 in *Hafnia alvei* 5-5 was determined. The promoter region was located at the upstream of inserted 0.8 kb *Sma* I fragment of plasmid 4.8 kb and detected by b-galactosidase assay. The plasmid with the putative promoters were nickel inducible. Five open reading frames were assigned to five polypeptides which were expressed from this determinant in *Escherichia coli*. The roles of the polypeptides from the open reading frames were analyzed with transposon mutagenesis.